In the United States Patent Office

In re Application of

Juergen Bode, Jost Seibler and Dirk Schuebeler

Serial-No. 09/841,843
Filed: April 25, 2001
Title: Method for marker-free repetitive DNA expression of cassette exchange in the genome of cells or parts of cells

DECLARATION

1. I, Alfred Nordheim, residing in Auf der Morgenstelle 15, 72076 Tuebingen, Germany, am a citizen of the Federal Republic of Germany. I am Professor for Molecular Biuology at the Institute for Cell Biology at the University of Tuebingen. My curriculum vitae is enclosed. I am trained and skilled in the field of stem cell research, in particular embryonic stem cells (ES cells). I have done extensive research in the field of homologous and site-specific recombination experiments in various cell types. A publication list is enclosed.

   I have carefully read and fully understood the US patent application of Juergen Bode, Jost Seibler and Dirk Schuebeler, US Serial No. 09/841,843 filed on April 25, 2001. Additionally, I have studied and fully understood the prior art references

   i) Thomas Schlake and Juergen Bode, 1994 Biochemistry, 33:12746-12751 "Us of Mutated FLP Recognition Target

(FRT) Sites for the Exchange of Expression Cassettes at Defined Chromosomal Loci";

ii) Ludwig *et al.*, 1996, Transgenic Research 5:385-395 "FLP-mediated site-specific recombination in microinjected murine zygotes"; and

iii) Jung *et al.*, 1993, Science 259: 984-987 "Shutdow of Class Switch Recombination by Deletion of a Switch Region Control Element"

3. The invention described in the US patent application 09/841,843 concerns a method for the recombinase mediated cassette exchange (RMCE), which allows the exchange of a first DNA cassette against a second incoming DNA cassette. The exchange is mediated by FLP recombinase in conjunction with sets of heterospecific FLP recombinase target (FRT) sequences (page 4, third paragraph of the application).

The application uses embryonic stem cells (ES cells) (page 5, second paragraph and page 8, first paragraph of the application).

For RMCE, the application uses a positive/negative selection strategy based on neomycin for positive selection and ganciclovir (GANC) for negative selection. For tagging the chromosomal loci, the target construct F_3*hygtk*F (positive selection for hygromycin resistance) and F_3*neo*F or F_3P^{pgk}*neo*F (for negative selection with ganciclovir) were used (page 8, third paragraph and page 9, second paragraph of the application).

In order to reduce the accumulation of inactivated clones prior to negative selection, the application features the combined positive and negative selection, and the positive selection is maintained during cultivation prior to the negative selection (page 13, paragraph following table 2 of the application). The application reports a targeting efficiency for positive plus negative double selection in the extraordinary high range of 54 and 100 % (page 15, first paragraph and table 3 of the application).

4. The prior art reference (i) Schlake and Bode 1994 Biochemistry 33:12746-12751 deals with an FLP/FRT system for site-specific recombination in cultured mammalian cells. Schlake and Bode were aware of the problems connected with homologous recombination occurring in embryonic stem cells and therefore chose to use established cultured mammalian cells (BHK or CV-1 cells) because they have lost the potential to perform homologous recombination. According to the understanding of Schlake and Bode especially BHK cells have a long track record for the safe production of vaccines (Schlake and Bode, page 12746, left column, first paragraph).

Schlake and Bode used hygromycin B and ganciclovir for selection (page 12747, left column, fourth paragraph), however, selection conditions are not described as set to maintain the positive selection by hygromycin all the time until the exchange of the first DNA expression cassette against the second incoming DNA expression cassette is completed.

Schlake and Bode use the plasmids F5HygTkF and F5NeoF for their experiments (page 12746, right column, second but last paragraph and page 12747, figure 1).

Schlake and Bode tested different plasmids with respect to their recombination efficiency and received enormous differences when different spacers (F_n, n being 0, 1, 2, 3, 4 or 5, page 12748, right column, table 1). Strikingly, the use of F_3 spacer led to an efficiency of only 0,24 (0,51) %, is a conf interval of only 1,5 %. In accordance with these experimental results, Schlake and Bode observed an increasing discrimination of the mutated spacer in the order of $F_1 < F_4 = F_2 < F_3 < F_5$ (page 12749, right column, first paragraph) and Schlake and Bode point to the fact that these observations leave unresolved the question as to the extend of F_n X F_n self-recognition. They further point out, that the signal strength in another experiment decreases in the order of $F >> F_1 > F_4 > F_2 > F_3 > F_5$, which reproduces the conclusions from table 1 (page 12750, left column, first paragraph). From these experiments it can be gained that F_3 as the omitted spacer which was used in the application does not seemed be of any particular advantage. The overall efficiency of the experiments reported by Schlake and Bode with omitted spacer, were 1,7 - 0,15 % (page 12749, paragraph bridging left and right column), which is significantly less to the reported efficiencies of the application of 54 and 100 % respectively (page 15, table 3).

5. When comparing the teaching of Schlake and Bode and the present application, significant differences in the disclosure are apparent. These are summarized in the following table.

	Invention US application 09/841,843	Schlake and Bode Biochemistry 1994 33:12746 - 12751
Cells	Embryonic stem cells	BHK and CV-1 cells, embryonic stem cells are excluded because cells with no potential for homologous recombination should be used (page 12746, left column, first paragraph)
Hygromcyin and neomycin constructs	F_3hygtkF and F_3NeoF (page 8, third paragraph and page 9, second paragraph)	F5HygtkF and F5NeoF (page 12747, figure 1)
Selection conditions	Maintenance of conditions for positive selection until the integration of the second DNA expression cassette is completed (claim 1 of the application)	
Efficiency	Targeting frequency F18, F21 and F22 is 100 %, 54 %, 100 % respectively (table 3, page 15)	Targeting frequency for F_3 is 0,24, 0,51 and 1,5 % respectively (table 1, page 12748)

For a person skilled in the art the teaching of the present invention is entirely surprising since the prior art teaches very low recombination efficiencies while the application achieves very high efficiencies at three different loci and is therefore a significant improvement over the prior art.

6. The prior art reference of Ludwig *et al.* 1996, Transgenic Research 5:385-395 reports that introduction of a FLP-

recombinase expression vector into transgenic one-cell fertilized mouse eggs induces a recombination event at a chromosomal FRT target locus (see abstract on page 385). Ludwig *et al.* discloses a classical FLP-mediated site-specific recombination between tandem arrays of FRT sites (page 393, figure 6). The frequency of recombination is not explicitly given, but it is merely referred to as "detectable" (page 393, left column, last line). The reference of Ludwig *et al.* therefore falls into the category of the prior art references mentioned in the application on page 3, third paragraph, as it also leaves behind a selectable marker and potentially unwanted vector sequences which are also inserted. Therefore, even though Ludwig *et al.* use mouse embryos, it is a prior art reference with the draw backs mentioned in the introduction of the application.

7. The reference of Jung *et al.* 1993, Science 259:984-987 refers to the class switching of immunoglobulins mediated by a homologous recombination event. It allows B cells to sequentially express antibodies that have identical specificities but that differ in class and thus effector function (page 984, left column, first few lines and right column, second paragraph). This study uses murine embryonic stem cells for the homologous recombination event. Consecutively the new cassette was deleted by the FLT recombination system, which is a site-specific deletion. As a result, the targeted locus retains a single copy of a FRT signal (page 965, left column, first paragraph).

Therefore, even though Jung *et al.* use embryonic stem cells, the system for recombination are the classical homologous recombination and the site-specific recombination which are described in detail on pages 1-3

of the present application. In particular, it is explicitly mentioned in Jung *et al.* that vector sequences are left behind in the genome, which is one of the goals, the present invention circumvents.

8. Combining the teachings of Schlake and Bode with either Ludwig *et al.* or Jung *et al.*, the person skilled in the art would not reach the claimed invention. The prior art references Ludwig *et al.* and Jung *et al.* use embryonic stem cells and murine embryos, respectively, the technology disclosed in these references is either classical site-specific recombination and/or homologous recombination, which all comprise major draw backs discussed in the invention. Therefore, a person skilled in the art, when starting from the disclosure of Schlake and Bode would not gain any further information from Jung *et al.* or Ludwig *et al.* in order to reach the claimed invention. In particular, a person skilled in the art would not be able to solve the problem of integrated vector sequences or low efficiencies in recombination together with the requirement for an incoming selectable marker.

Therefore, it is believed that the teaching of the present invention is not obvious from the prior art references.

9. I further declare, that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements are made with the knowledge that willful false statements and the like, so made, are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the US Code, and that such willful false statements my jeopardize the validity of the application or any patent issuing thereon.

Tübingen Jan. 20, 2004 C. Nordheim

Date and Signature

Enclosures:
Curriculum Vitae
Publication List

Prof. Dr. Alfred Nordheim - Publications

1 Timmis, K. N., Cabello, F., Andres, I., Nordheim, A., Burkhardt, H. J., and Cohen, S. N. (1978). Instability of plasmid DNA sequences: macro and micro evolution of the antibiotic resistance plasmid R6-5. Mol Gen Genet 167, 11-19.

2 Synenki, R. M., Nordheim, A., and Timmis, K. N. (1979). Plasmid replication functions. III. Origin and direction of replication of a "mini" plasmid derived from R6-5. Mol Gen Genet 168, 27-36.

3 Nordheim, A., Hashimoto-Gotoh, T., and Timmis, K. N. (1980). Location of two relaxation nick sites in R6K and single sites in pSC101 and RSF1010 close to origins of vegetative replication: implication for conjugal transfer of plasmid deoxyribonucleic acid. J Bacteriol 144, 923-932.

4 Hashimoto-Gotoh, T., Franklin, F. C., Nordheim, A., and Timmis, K. N. (1981). Specific-purpose plasmid cloning vectors. I. Low copy number, temperature-sensitive, mobilization-defective pSC101-derived containment vectors. Gene 16, 227-235.

5 Lafer, E. M., Moller, A., Nordheim, A., Stollar, B. D., and Rich, A. (1981). Antibodies specific for left-handed Z-DNA. Proc Natl Acad Sci U S A 78, 3546-3550.

6 Moller, A., Nordheim, A., Nichols, S. R., and Rich, A. (1981). 7-Methylguanine in poly(dG-dC).poly(dG-dC) facilitates z-DNA formation. Proc Natl Acad Sci U S A 78, 4777-4781.

7 Nordheim, A., Pardue, M. L., Lafer, E. M., Moller, A., Stollar, B. D., and Rich, A. (1981). Antibodies to left-handed Z-DNA bind to interband regions of Drosophila polytene chromosomes. Nature 294, 417-422.

8 Moller, A., Gabriels, J. E., Lafer, E. M., Nordheim, A., Rich, A., and Stollar, B. D. (1982). Monoclonal antibodies recognize different parts of Z-DNA. J Biol Chem 257, 12081-12085.

9 Nordheim, A., Lafer, E. M., Peck, L. J., Wang, J. C., Stollar, B. D., and Rich, A. (1982). Negatively supercoiled plasmids contain left-handed Z-DNA segments as detected by specific antibody binding. Cell 31, 309-318.

10 Nordheim, A., Tesser, P., Azorin, F., Kwon, Y. H., Moller, A., and Rich, A. (1982). Isolation of Drosophila proteins that bind selectively to left-handed Z-DNA. Proc Natl Acad Sci U S A 79, 7729-7733.

11 Nordheim, A., Lafer, E. M., Moller, A., Stollar, B. D., Pardue, M. L., and Rich, A. (1982). Left-handed Z-DNA: a DNA structure with biological function? Prog Clin Biol Res 85 Pt A, 101-113.

12 Patel, D. J., Kozlowski, S. A., Nordheim, A., and Rich, A. (1982). Right-handed and left-handed DNA: studies of B- and Z-DNA by using proton nuclear Overhauser effect and P NMR. Proc Natl Acad Sci U S A 79, 1413-1417.

13 Peck, L. J., Nordheim, A., Rich, A., and Wang, J. C. (1982). Flipping of cloned d(pCpG)n.d(pCpG)n DNA sequences from right- to left-handed helical structure by salt, Co(III), or negative supercoiling. Proc Natl Acad Sci U S A 79, 4560-4564.

14 Santella, R. M., Grunberger, D., Nordheim, A., and Rich, A. (1982). N-2-Acetylaminofluorene modification of poly(dG-m5dC) . poly(dG-m5dC) induces the Z-DNA conformation. Biochem Biophys Res Commun 106, 1226-1232.

15 Azorin, F., Nordheim, A., and Rich, A. (1983). Formation of Z-DNA in negatively supercoiled plasmids is sensitive to small changes in salt concentration within the physiological range. Embo J 2, 649-655.

Prof. Dr. Alfred Nordheim - Publications

16 Lafer, E. M., Valle, R. P., Moller, A., Nordheim, A., Schur, P. H., Rich, A., and Stollar, B. D. (1983). Z-DNA-specific antibodies in human systemic lupus erythematosus. J Clin Invest 71, 314-321.

17 Lafer, E. M., Moller, A., Valle, R. P., Nordheim, A., Rich, A., and Stollar, B. D. (1983). Antibody recognition of Z-DNA. Cold Spring Harb Symp Quant Biol 47 Pt 1, 155-162.

18 Lipps, H. J., Nordheim, A., Lafer, E. M., Ammermann, D., Stollar, B. D., and Rich, A. (1983). Antibodies against Z DNA react with the macronucleus but not the micronucleus of the hypotrichous ciliate stylonychia mytilus. Cell 32, 435-441.

19 Nordheim, A., and Rich, A. (1983). Negatively supercoiled simian virus 40 DNA contains Z-DNA segments within transcriptional enhancer sequences. Nature 303, 674-679.

20 Nordheim, A., and Rich, A. (1983). The sequence (dC-dA)n X (dG-dT)n forms left-handed Z-DNA in negatively supercoiled plasmids. Proc Natl Acad Sci U S A 80, 1821-1825.

21 Nordheim, A., Hao, W. M., Wogan, G. N., and Rich, A. (1983). Salt-induced conversion of B-DNA to Z-DNA inhibited by aflatoxin B1. Science 219, 1434-1436.

22 Nordheim, A., Peck, L. J., Lafer, E. M., Stollar, B. D., Wang, J. C., and Rich, A. (1983). Supercoiling and left-handed Z-DNA. Cold Spring Harb Symp Quant Biol 47 Pt 1, 93-100.

23 Pardue, M. L., Nordheim, A., Lafer, E. M., Stollar, B. D., and Rich, A. (1983). Z-DNA and the polytene chromosome. Cold Spring Harb Symp Quant Biol 47 Pt 1, 171-176.

24 Rich, A., Nordheim, A., and Azorin, F. (1983). Stabilization and detection of natural left-handed Z-DNA. J Biomol Struct Dyn 1, 1-19.

25 Moller, A., Nordheim, A., Kozlowski, S. A., Patel, D. J., and Rich, A. (1984). Bromination stabilizes poly(dG-dC) in the Z-DNA form under low-salt conditions. Biochemistry 23, 54-62.

26 Rich, A., Nordheim, A., and Wang, A. H. (1984). The chemistry and biology of left-handed Z-DNA. Annu Rev Biochem 53, 791-846.

27 Schroter, H., Maier, G., Ponstingl, H., and Nordheim, A. (1985). DNA intercalators induce specific release of HMG 14, HMG 17 and other DNA-binding proteins from chicken erythrocyte chromatin. Embo J 4, 3867-3872.

28 Stollar, B. D., Lafer, E., Nordheim, A., Pardue, M. L., and Rich, A. (1985). Immunological probes for the biological significance of Z-DNA. Prog Clin Biol Res 172B, 323-332.

29 Darby, M. K., Herrera, R. E., Vosberg, H. P., and Nordheim, A. (1986). DNA topoisomerase II cleaves at specific sites in the 5' flanking region of c-fos proto-oncogenes in vitro. Embo J 5, 2257-2265.

30 Nordheim, A., Pardue, M. L., Weiner, L. M., Lowenhaupt, K., Scholten, P., Moller, A., Rich, A., and Stollar, B. D. (1986). Analysis of Z-DNA in fixed polytene chromosomes with monoclonal antibodies that show base sequence-dependent selectivity in reactions with supercoiled plasmids and polynucleotides. J Biol Chem 261, 468-476.

31 Peck, L. J., Wang, J. C., Nordheim, A., and Rich, A. (1986). Rate of B to Z structural transition of supercoiled DNA. J Mol Biol 190, 125-127.

Prof. Dr. Alfred Nordheim - Publications

32 Runkel, L., and Nordheim, A. (1986). Conformational DNA transition in the in vitro torsionally strained chicken beta-globin 5' region. Nucleic Acids Res 14, 7143-7158.

33 Runkel, L., and Nordheim, A. (1986). Chemical footprinting of the interaction between left-handed Z-DNA and anti-Z-DNA antibodies by diethylpyrocarbonate carbethoxylation. J Mol Biol 189, 487-501.

34 Scholten, P. M., and Nordheim, A. (1986). Diethyl pyrocarbonate: a chemical probe for DNA cruciforms. Nucleic Acids Res 14, 3981-3993.

35 Nordheim, A., Herrera, R. E., and Rich, A. (1987). Binding of anti-Z-DNA antibodies to negatively supercoiled SV40 DNA. Nucleic Acids Res 15, 1661-1677.

36 Pardue, M. L., Lowenhaupt, K., Rich, A., and Nordheim, A. (1987). (dC-dA)n.(dG-dT)n sequences have evolutionarily conserved chromosomal locations in Drosophila with implications for roles in chromosome structure and function. Embo J 6, 1781-1789.

37 Schroter, H., Shaw, P. E., and Nordheim, A. (1987). Purification of intercalator-released p67, a polypeptide that interacts specifically with the c-fos serum response element. Nucleic Acids Res 15, 10145-10158.

38 Kramer, H., Amouyal, M., Nordheim, A., and Muller-Hill, B. (1988). DNA supercoiling changes the spacing requirement of two lac operators for DNA loop formation with lac repressor. Embo J 7, 547-556.

39 Nordheim, A., and Meese, K. (1988). Topoisomer gel retardation: detection of anti-Z-DNA antibodies bound to Z-DNA within supercoiled DNA minicircles. Nucleic Acids Res 16, 21-37.

40 Vingron, M., Nordheim, A., and Muller, R. (1988). Anatomy of fos proteins. Oncogene Res 3, 1-7.

41 Herrera, R. E., Shaw, P. E., and Nordheim, A. (1989). Occupation of the c-fos serum response element in vivo by a multi-protein complex is unaltered by growth factor induction. Nature 340, 68-70.

42 Schroter, H., and Nordheim, A. (1989). Silver-stained protein gels allow recovery of transcription factors still active in specific DNA-binding. Nucleic Acids Res 17, 6415.

43 Shaw, P. E., Frasch, S., and Nordheim, A. (1989). Repression of c-fos transcription is mediated through p67SRF bound to the SRE. Embo J 8, 2567-2574.

44 Shaw, P. E., Schroter, H., and Nordheim, A. (1989). The ability of a ternary complex to form over the serum response element correlates with serum inducibility of the human c-fos promoter. Cell 56, 563-572.

45 Schroter, H., Mueller, C. G., Meese, K., and Nordheim, A. (1990). Synergism in ternary complex formation between the dimeric glycoprotein p67SRF, polypeptide p62TCF and the c-fos serum response element. Embo J 9, 1123-1130.

46 Stewart, A. F., Herrera, R. E., and Nordheim, A. (1990). Rapid induction of c-fos transcription reveals quantitative linkage of RNA polymerase II and DNA topoisomerase I enzyme activities. Cell 60, 141-149.

47 Droge, P., and Nordheim, A. (1991). Transcription-induced conformational change in a topologically closed DNA domain. Nucleic Acids Res 19, 2941-2946.

48 Hipskind, R. A., Rao, V. N., Mueller, C. G., Reddy, E. S., and Nordheim, A. (1991). Ets-related protein Elk-1 is homologous to the c-fos regulatory factor p62TCF. Nature 354, 531-534.

49 Hipskind, R. A., and Nordheim, A. (1991). In vitro transcriptional analysis of the human c-fos proto-oncogene. J Biol Chem 266, 19572-19582.

50 Hipskind, R. A., and Nordheim, A. (1991). Functional dissection in vitro of the human c-fos promoter. J Biol Chem 266, 19583-19592.

51 Janknecht, R., de Martynoff, G., Lou, J., Hipskind, R. A., Nordheim, A., and Stunnenberg, H. G. (1991). Rapid and efficient purification of native histidine-tagged protein expressed by recombinant vaccinia virus. Proc Natl Acad Sci U S A 88, 8972-8976.

52 Mueller, C. G., and Nordheim, A. (1991). A protein domain conserved between yeast MCM1 and human SRF directs ternary complex formation. Embo J 10, 4219-4229.

53 Runkel, L., Shaw, P. E., Herrera, R. E., Hipskind, R. A., and Nordheim, A. (1991). Multiple basal promoter elements determine the level of human c-fos transcription. Mol Cell Biol 11, 1270-1280.

54 Schumacher, A., Schroter, H., Multhaup, G., and Nordheim, A. (1991). Murine cyclophilin-S1: a variant peptidyl-prolyl isomerase with a putative signal sequence expressed in differentiating F9 cells. Biochim Biophys Acta 1129, 13-22.

55 Droge, P., and Nordheim, A. (1992). Topoisomer gel retardation: protein recognition of torsional stress-induced DNA conformations. Methods Enzymol 212, 371-387.

56 Janknecht, R., and Nordheim, A. (1992). Affinity purification of histidine-tagged proteins transiently produced in HeLa cells. Gene 121, 321-324.

57 Janknecht, R., and Nordheim, A. (1992). Elk-1 protein domains required for direct and SRF-assisted DNA-binding. Nucleic Acids Res 20, 3317-3324.

58 Janknecht, R., Hipskind, R. A., Houthaeve, T., Nordheim, A., and Stunnenberg, H. G. (1992). Identification of multiple SRF N-terminal phosphorylation sites affecting DNA binding properties. Embo J 11, 1045-1054.

59 Schumacher, A., and Nordheim, A. (1992). Progress towards a molecular understanding of cyclosporin A-mediated immunosuppression. Clin Investig 70, 773-779.

60 Janknecht, R., and Nordheim, A. (1993). Gene regulation by Ets proteins. Biochim Biophys Acta 1155, 346-356.

61 Janknecht, R., Ernst, W. H., Pingoud, V., and Nordheim, A. (1993). Activation of ternary complex factor Elk-1 by MAP kinases. Embo J 12, 5097-5104.

62 Janknecht, R., Ernst, W. H., Houthaeve, T., and Nordheim, A. (1993). C-terminal phosphorylation of the serum-response factor. Eur J Biochem 216, 469-475.

63 Zinck, R., Hipskind, R. A., Pingoud, V., and Nordheim, A. (1993). c-fos transcriptional activation and repression correlate temporally with the phosphorylation status of TCF. Embo J 12, 2377-2387.

64 Cahill, M. A., Nordheim, A., and Janknecht, R. (1994). Co-occurrence of CArG boxes and TCF sites within viral genomes. Biochem Biophys Res Commun 205, 545-551.

65 Cahill, M. A., Ernst, W. H., Janknecht, R., and Nordheim, A. (1994). Regulatory squelching. FEBS Lett 344, 105-108.

66 Hipskind, R. A., Baccarini, M., and Nordheim, A. (1994). Transient activation of RAF-1, MEK, and ERK2 coincides kinetically with ternary complex factor phosphorylation and immediate-early gene promoter activity in vivo. Mol Cell Biol 14, 6219-6231.

67 Hipskind, R. A., Buscher, D., Nordheim, A., and Baccarini, M. (1994). Ras/MAP kinase-dependent and -independent signaling pathways target distinct ternary complex factors. Genes Dev 8, 1803-1816.

68 Janknecht, R., Zinck, R., Ernst, W. H., and Nordheim, A. (1994). Functional dissection of the transcription factor Elk-1. Oncogene 9, 1273-1278.

69 Janz, M., Lehmann, U., Olde Weghuis, D., de Leeuw, B., Geurts van Kessel, A., Gilgenkrantz, S., Hipskind, R. A., and Nordheim, A. (1994). Refined mapping of the human Ets-related gene Elk-1 to Xp11.2-p11.4, distal to the OATL1 region. Hum Genet 94, 442-444.

70 Nordheim, A. (1994). Transcription factors. CREB takes CBP to tango. Nature 370, 177-178.

71 Pingoud, V., Zinck, R., Hipskind, R. A., Janknecht, R., and Nordheim, A. (1994). Heterogeneity of ternary complex factors in HeLa cell nuclear extracts. J Biol Chem 269, 23310-23317.

72 Sachsenmaier, C., Radler-Pohl, A., Zinck, R., Nordheim, A., Herrlich, P., and Rahmsdorf, H. J. (1994). Involvement of growth factor receptors in the mammalian UVC response. Cell 78, 963-972.

73 Schumacher, A., Westermann, B., Osborn, M., and Nordheim, A. (1994). The N-terminal signal peptide of the murine cyclophilin mCyP-S1 is required in vivo for ER localization. Eur J Cell Biol 63, 182-191.

74 Althoefer, H., Schleiffer, A., Wassmann, K., Nordheim, A., and Ammerer, G. (1995). Mcm1 is required to coordinate G2-specific transcription in Saccharomyces cerevisiae. Mol Cell Biol 15, 5917-5928.

75 Ernst, W. H., Janknecht, R., Cahill, M. A., and Nordheim, A. (1995). Transcriptional repression mediated by the serum response factor. FEBS Lett 357, 45-49.

76 Janknecht, R., Ernst, W. H., and Nordheim, A. (1995). SAP1a is a nuclear target of signaling cascades involving ERKs. Oncogene 10, 1209-1216.

77 Janknecht, R., Cahill, M. A., and Nordheim, A. (1995). Signal integration at the c-fos promoter. Carcinogenesis 16, 443-450.

78 Janz, M., De Leeuw, B., Weghuis, D. O., Werner, M., Nolte, M., Geurts Van Kessel, A., Nordheim, A., and Hipskind, R. A. (1995). Interphase cytogenetic analysis of distinct X-chromosomal translocation breakpoints in synovial sarcoma. J Pathol 175, 391-396.

79 Zinck, R., Cahill, M. A., Kracht, M., Sachsenmaier, C., Hipskind, R. A., and Nordheim, A. (1995). Protein synthesis inhibitors reveal differential regulation of mitogen-activated protein kinase and stress-activated protein kinase pathways that converge on Elk-1. Mol Cell Biol 15, 4930-4938.

80 Cahill, M. A., Nordheim, A., and Xu, Y. Z. (1996). Crosslinking of SRF to the c-fos SRE CArG box guanines using photo-active thioguanine oligodeoxynucleotides. Biochem Biophys Res Commun 229, 170-175.

Prof. Dr. Alfred Nordheim - Publications

81 Cahill, M. A., Peter, M. E., Kischkel, F. C., Chinnaiyan, A. M., Dixit, V. M., Krammer, P. H., and Nordheim, A. (1996). CD95 (APO-1/Fas) induces activation of SAP kinases downstream of ICE-like proteases. Oncogene 13, 2087-2096.

82 Cahill, M. A., Janknecht, R., and Nordheim, A. (1996). Signalling pathways: jack of all cascades. Curr Biol 6, 16-19.

83 Janknecht, R., and Nordheim, A. (1996). MAP kinase-dependent transcriptional coactivation by Elk-1 and its cofactor CBP. Biochem Biophys Res Commun 228, 831-837.

84 Janknecht, R., and Nordheim, A. (1996). Regulation of the c-fos promoter by the ternary complex factor Sap-1a and its coactivator CBP. Oncogene 12, 1961-1969.

85 Muller, J. M., Cahill, M. A., Nordheim, A., and Baeuerle, P. A. (1996). The transcription factor TCF/Elk-1: a nuclear sensor of changes in the cellular redox status. Adv Exp Med Biol 387, 77-84.

86 Bredemeier-Ernst, I., Nordheim, A., and Janknecht, R. (1997). Transcriptional activity and constitutive nuclear localization of the ETS protein Elf-1. FEBS Lett 408, 47-51.

87 Herrera, R. E., Nordheim, A., and Stewart, A. F. (1997). Chromatin structure analysis of the human c-fos promoter reveals a centrally positioned nucleosome. Chromosoma 106, 284-292.

88 Malek, N. P., Casper, J., Looijenga, L. H., Strohmeyer, T., Schmoll, H. J., Nordheim, A., and Janknecht, R. (1997). Quantification of additional short arms of chromosome 12 in germ cell tumours using the polymerase chain reaction. Eur J Cancer 33, 1488-1494.

89 Muller, J. M., Cahill, M. A., Rupec, R. A., Baeuerle, P. A., and Nordheim, A. (1997). Antioxidants as well as oxidants activate c-fos via Ras-dependent activation of extracellular-signal-regulated kinase 2 and Elk-1. Eur J Biochem 244, 45-52.

90 Ruhlmann, A., and Nordheim, A. (1997). Effects of the immunosuppressive drugs CsA and FK506 on intracellular signalling and gene regulation. Immunobiology 198, 192-206.

91 Arsenian, S., Weinhold, B., Oelgeschlager, M., Ruther, U., and Nordheim, A. (1998). Serum response factor is essential for mesoderm formation during mouse embryogenesis. Embo J 17, 6289-6299.

92 Dittmer, J., and Nordheim, A. (1998). Ets transcription factors and human disease. Biochim Biophys Acta 1377, F1-11.

93 Panitz, F., Krain, B., Hollemann, T., Nordheim, A., and Pieler, T. (1998). The Spemann organizer-expressed zinc finger gene Xegr-1 responds to the MAP kinase/Ets-SRF signal transduction pathway. Embo J 17, 4414-4425.

94 Ahlers, C., Kreideweiss, S., Nordheim, A., and Ruhlmann, A. (1999). Cyclosporin A inhibits Ca2+-mediated upregulation of the DNA repair enzyme DNA polymerase beta in human peripheral blood mononuclear cells. Eur J Biochem 264, 952-959.

95 Blumenthal, S. G., Aichele, G., Wirth, T., Czernilofsky, A. P., Nordheim, A., and Dittmer, J. (1999). Regulation of the human interleukin-5 promoter by Ets transcription factors. Ets1 and Ets2, but not Elf-1, cooperate with GATA3 and HTLV-I Tax1. J Biol Chem 274, 12910-12916.

96 Heidenreich, O., Neininger, A., Schratt, G., Zinck, R., Cahill, M. A., Engel, K., Kotlyarov, A., Kraft, R., Kostka, S., Gaestel, M., and Nordheim, A. (1999). MAPKAP kinase 2 phosphorylates serum response factor in vitro and in vivo. J Biol Chem 274, 14434-14443.

97 Hollnagel, A., Oehlmann, V., Heymer, J., Ruther, U., and Nordheim, A. (1999). Id genes are direct targets of bone morphogenetic protein induction in embryonic stem cells. J Biol Chem 274, 19838-19845.

98 Krain, B., and Nordheim, A. (1999). Artefactual gene induction during preparation of Xenopus laevis animal cap explants. Int J Dev Biol 43, 563-566.

99 Kreideweiss, S., Ahlers, C., Nordheim, A., and Ruhlmann, A. (1999). Ca2+-induced p38/SAPK signalling inhibited by the immunosuppressant cyclosporin A in human peripheral blood mononuclear cells. Eur J Biochem 265, 1075-1084.

100 Lehmann, U., Brocke, P., Dittmer, J., and Nordheim, A. (1999). Characterization of the human elk-1 promoter. Potential role of a downstream intronic sequence for elk-1 gene expression in monocytes. J Biol Chem 274, 1736-1744.

101 Vuong, G. L., Weiss, S. M., Kammer, W., Priemer, M., Vingron, M., Nordheim, A., and Cahill, M. A. (2000). Improved sensitivity proteomics by postharvest alkylation and radioactive labelling of proteins. Electrophoresis 21, 2594-2605.

102 Weinhold, B., Schratt, G., Arsenian, S., Berger, J., Kamino, K., Schwarz, H., Ruther, U., and Nordheim, A. (2000). Srf(-/-) ES cells display non-cell-autonomous impairment in mesodermal differentiation. Embo J 19, 5835-5844.

103 White, C. M., Heidenreich, O., Nordheim, A., and Beerman, T. A. (2000). Evaluation of the effectiveness of DNA-binding drugs to inhibit transcription using the c-fos serum response element as a target. Biochemistry 39, 12262-12273.

104 Boitchenko, V., Kuprash, D., Nordheim, A., Ruhlmann, A., and Nedospasov, S. (2001). Cyclosporin A Blocks PMA and Ionomycin Activated Lymphotoxin Expression in a
Human T-Cell Line. Russ J Immunol 6, 9-16.

105 Lindemann, R. K., Ballschmieter, P., Nordheim, A., and Dittmer, J. (2001). Transforming growth factor beta regulates parathyroid hormone-related protein expression in MDA-MB-231 breast cancer cells through a novel Smad/Ets synergism. J Biol Chem 276, 46661-46670.

106 Nentwich, O., Munchberg, F. E., Frommer, G., and Nordheim, A. (2001). Tissue-specific expression of the Ets gene Xsap-1 during Xenopus laevis development. Mech Dev 109, 433-436.

107 Schratt, G., Weinhold, B., Lundberg, A. S., Schuck, S., Berger, J., Schwarz, H., Weinberg, R. A., Ruther, U., and Nordheim, A. (2001). Serum response factor is required for immediate-early gene activation yet is dispensable for proliferation of embryonic stem cells. Mol Cell Biol 21, 2933-2943.

108 Szyrach, M., Munchberg, F. E., Riehle, H., Nordheim, A., Krauter, J., Nagel, S., Heil, G., and Heidenreich, O. (2001). Cleavage of AML1/MTG8 by asymmetric hammerhead ribozymes. Eur J Biochem 268, 3550-3557.

109 Kuprash, D. V., Boitchenko, V. E., Yarovinsky, F. O., Rice, N. R., Nordheim, A., Ruhlmann, A., and Nedospasov, S. A. (2002). Cyclosporin A blocks the expression of lymphotoxin alpha, but not lymphotoxin beta, in human peripheral blood mononuclear cells. Blood 100, 1721-1727.

110 Schratt, G., Philippar, U., Berger, J., Schwarz, H., Heidenreich, O., and Nordheim, A. (2002). Serum response factor is crucial for actin cytoskeletal organization and focal adhesion assembly in embryonic stem cells. J Cell Biol 156, 737-750.

111 Wang, D. Z., Li, S., Hockemeyer, D., Sutherland, L., Wang, Z., Schratt, G., Richardson, J. A., Nordheim, A., and Olson, E. N. (2002). Potentiation of serum response factor activity by a family of myocardin-related transcription factors. Proc Natl Acad Sci U S A 99, 14855-14860.

112 Wiebel, F. F., Rennekampff, V., Vintersten, K., and Nordheim, A. (2002). Generation of mice carrying conditional knockout alleles for the transcription factor SRF. Genesis 32, 124-126.

113 Ballschmieter, P., Braig, M., Lindemann, R. K., Nordheim, A., and Dittmer, J. (2003). Splicing variant DeltaVII-Ets1 is downregulated in invasive Ets1-expressing breast cancer cells. Int J Oncol 22, 849-853.

114 Heidenreich, O., Krauter, J., Riehle, H., Hadwiger, P., John, M., Heil, G., Vornlocher, H., and Nordheim, A. (2003). AML1/MTG8 oncogene suppression by small interfering RNAs supports myeloid differentiation of t(8;21)-positive leukemic cells. Blood 15;101, 3157-3163.

115 Lindemann, R. K., Braig, M., Ballschmieter, P., Guise, T. A., Nordheim, A., and Dittmer, J. (2003). Protein kinase Calpha regulates Ets1 transcriptional activity in invasive breast cancer cells. Int J Oncol 22, 799-805.

116 Lindemann, R. K., Braig, M., Hauser, C. A., Nordheim, A., and Dittmer, J. (2003). Ets2 and protein kinase C-epsilon are important regulators of parathyroid hormone-related protein expression in MCF-7 breast cancer cells. Biochem J 372, 787-797.

117 Lindemann, R. K., Nordheim, A., and Dittmer, J. (2003). Interfering with TGFbeta-induced Smad3 nuclear accumulation differentially affects TGFbeta-dependent gene expression. Molecular Cancer 2, 10 pages.

118 Schuck, S., Soloaga, A., Schratt, G., Arthur, J. S., and Nordheim, A. (2003). The kinase MSK1 is required for induction of c-fos by lysophosphatidic acid in mouse embryonic stem cells. BMC Mol Biol 4, 6.

119 Cesari, F., Brecht, S., Vintersten, K., Vuong, L. G., Hofmann, M., Klingel, K., Schnorr, J. J., Arsenian, S., Schild, H., Herdegen, T., et al. (2004). Mice deficient for the ets transcription factor elk-1 show normal immune responses and mildly impaired neuronal gene activation. Mol Cell Biol 24, 294-305.

120 Cesari, F., Rennekampff, V., Vintersten, K., Vuong, G. L., Bode, J., Wiebel, F. F., and Nordheim, A. (submitted). Site-directed mutagenesis of the genomic Elk-1 locus by Recombinase Mediated Cassette Exchange (RMCE).

121 Schratt, G., Philippar, U., Hockemeyer, D., Alberti, S., Schwarz, H., and Nordheim, A. (submitted). SRF regulates Bcl-2 expression and promotes cell survival during embryonic development.

Prof. Dr. Alfred Nordheim - Patents

1 Nordheim, A., and Rich, A. (1984). The use of structurally flexible DNA elements to regulate gene activity (MIT Patent, National Patent Office of the United States of America, Washington, USA).

2 Nordheim, A., Ernst, W., and Janknecht, R. (1993). Neue Transkriptionsfaktor-Mutanten und ihre Verwendung (Deutsches Patentamt München, Germany).

3 Nordheim, A., Hipskind, R. A., Pingoud, V., and Zinck, R. (1993). In vitro-Meßsystem zur Erkennung von Wirkstoffen, die Signalübertragung, Genaktivität und Wachstum eukaryonter Zellen beeinflussen (Deutsches Patentamt München, Germany).

4 Cahill, M. A., Drukier, A., and Nordheim, A. (1998). Methods and apparatus for the separation of components from a biological material (European Patent Application (A33383EP), PCT Patent application (A34519PCT), Europe).

5 Cahill, M. A., and Nordheim, A. (1999). Reversibly fixed gels for gel eletrophoresis (European Patent Application (A33890EP), PCT Patent Application (A34912PCT), Europe).

6 Cahill, M. A., Drukier, A., and Nordheim, A. (1999). Labelling of peptides and proteins (European Patent Application (A33389EP), PCT Patent Application (A34626PCT), Europe).

7 Cahill, M. A., Drukier, A., and Nordheim, A. (1999). Micropreparative isoelectric focussing (European Patent Application (A33384EP), PCT Patent Application (A34829PCT), Europe).

8 Nordheim, A. (2000). SRF as a regulator of cellular activities (European Patent Application (A35243EP), Europe).

9 Kammer, W., and Nordheim, A. (2002). Hängende Tropfen (Deutsche Patentanmeldung (10210908.7), Germany).

10 Nordheim, A., Kammer, W., Weiß, S., and Vuong, G. L. (2002). Verfahren zur Detektion von Biomolekülen (Deutsche Patentanmeldung (10243303.8), Germany).

CURRICULUM VITAE
Prof. Dr. Alfred Nordheim

Home Address:		Im Brennofen 14 72135 Dettenhausen Germany Tel. + Fax +49-7157-521233 e-mail: nordheim@supra-net.net
Work Address:		Eberhard-Karls-Universität Tübingen Institute for Cell Biology Department of Molecular Biology Auf der Morgenstelle 15 72076 Tübingen Germany Tel. +49-7071- 29-78898 Fax. +49-7071- 29-5359 e-mail: alfred.nordheim@uni-tuebingen.de
Date of Birth:		December 1, 1951
Place of Birth:		Philippsthal/W (Germany)
Nationality:		German
Family Status:		married
Education:	1972-76	Biology Major, Free University Berlin, Germany
	1975-76	Biology Major at the University College of North Wales, Bangor, Great Britain
	1976	Master of Science in Biology, Free University Berlin, Germany
	1977-79	Promotion to Dr. rer. nat. ("With Honors"), Max-Planck-Institute, Berlin-Dahlem, Germany
Scientific Training:	1980-83	Postdoctoral fellow with Prof. A. Rich, Massachusetts Institute of Technology (MIT) Department of Biology Cambridge, Mass., USA
Academic Degrees:	1976	Master of Science in Biology, Free University Berlin,Germany
	1979	Promotion to Dr. rer. nat., Free University Berlin, Germany
	1985	Habilitation + Venia Legendi, University of Heidelberg, Germany

	1989	Full Professor of Molecular Biology, Hannover Medical School, Germany
	1997	Full Profeessor of Molecular Biology, Tübingen University, Germany
Research and Teaching Experience:	1980-81	Postgraduate scientist, MIT, Cambridge, USA
	1982-83	Research Associate , MIT, Cambridge, USA
	1984-89	Head of research group, ZMBH, University of Heidelberg, Germany
	2000	Guest Professor (6 months), MIT, Whitehead Institute, Cambridge USA. Host: Prof. Dr. R. A. Weinberg
Scientific Administration:	1989-1996	Director of the Institute for Molecular Biology at Hannover Medical School, Germany
	1989-	"Speaker" for the DFG-Graduate College "Molecular Pathophysiology of Cell Growth"
	1992-	Deputy Speaker for the SFB 265 „Immune Reactions and Pathomechanisms during Organ Transplantation", Hannover, Germany
	1996-	Coordinator of the EC Network "Signalling Networks in Development and Disease" (TMR Network)
	1997-	Director of the Department of Molecular Biology, Interdisciplinary Insutute for Cell Biology, Eberhard-Karls-University of Tübingen.
Scientific Awards and Functions:	1991	Elected member of the "European Molecular Biology Organization (EMBO)"
	1992	Max-Planck-Research Prize of the Max-Planck Society and the Alexander von Humboldt Foundation (together with R.A. Weinberg, MIT, USA)
	1997-	Member of the Research Advisory Committee of the Medical Faculty at Eberhard-Karls-University of Tübingen
	1998-2001	Member of the Expert Advisory Board at the Max-Planck-Institute for Developmental Biology, Tübingen
	1999-	Member of the "Rector's Committee for Research Questions" at Eberhard-Karls-University of Tübingen

	2002-	Member of the Scientific Advisory Board of the Dr. Mildred Scheel Foundation for Cancer Research, Bonn, Germany
	2002-	Vice-President of the German Genetics Society (GfG)
Duties as Scientific Reviewer:	1986-	Reviewer for various national and international scientific organizations (Switzerland, Great Britain, USA, Germany) and for different scientific journals (Cell, EMBO Journal, Nature, Molecular & Cellular Biology, J. Mol. Biol., Nucleic Acids Research, etc.)
	1988-	Reviewer for grant applications, Schwerpunktprogramme and various SFB programs of the German Research Society (DFG)
	1992-	Austrian Funds for the Advancement of Scientific Research (FWF): Reviewer for various grant programs and the SFB 002 in Innsbruck (Austria)
	1992-1998	Appointment as scientific advisor to the German-Israeli Foundation for Scientific Research and Development (GIF)
	1995-	Reviewer for the German National Board of Academic Advisors ("Wissenschaftsrat") (Köln)
	1995-	Reviewer for the Volkswagen-Foundation
	1996-1999	Elected DFG Expert Reviewer for the subject of Cell Biology
Membership to scientific organizations:		-Society for Biological Chemistry (FEBS) -Society for Genetic Engineering -European Molecular Biology Organization (EMBO) -American Association for the Advancement of Sciences (AAAS) -"Förderverein Molekularbiologie und Biomedi: (Founding member, 1st president) -New York Academy of Sciences -German Association for Gene Therapy -Deutsche Krebsgesellschaft e.V. - German Society for Proteome Research
Founding of a company:	Feb. 2000	Founding of the company "ProteoMed GmbH", Tübingen; Position as CEO.
	Feb. 2001	Merger of "ProteoMed GmbH" with "ProteoSys GmbH" to form "ProteoSys AG", Mainz; Position as Scientific Advisor.